



TITLE:

高親和性インターロイキン2受容体の機能および構造に関する研究(Dissertation_全文)

AUTHOR(S):

Arima, Nobuyoshi

CITATION:

Arima, Nobuyoshi. 高親和性インターロイキン2受容体の機能および構造に関する研究. 京都大学, 1993, 博士(医学)

ISSUE DATE:

1993-03-23

URL:

<https://doi.org/10.11501/3066188>

RIGHT:

主論文は出版社の許諾条件により公開していない部分があります

②
高親和性インターロイキン2受容体の
機能および構造に関する研究

有馬 靖佳

0022-1767/91/14710-3396\$02.00/0

THE JOURNAL OF IMMUNOLOGY

Copyright © 1991 by The American Association of Immunologists

Vol. 147, 3396-3401, No. 10, November 15, 1991

Printed in U.S.A.

主
論
文
1

THE IL-2 RECEPTOR α -CHAIN ALTERS THE BINDING OF IL-2 TO THE β -CHAIN¹

NOBUYOSHI ARIMA,* MASANORI KAMIO,* MINORU OKUMA,* GRACE JU,[†] AND
TAKASHI UCHIYAMA^{2‡}

*From the *First Division of Internal Medicine, Faculty of Medicine, Kyoto University, 54 Shogoin-Kawaramachi, Sakyo, Kyoto 606, Japan; †Institute for Virus Research, Kyoto University, 53 Shogoin-Kawaramachi, Sakyo, Kyoto 606, Japan; and the ‡Department of Molecular Genetics, Roche Research Center, Hoffman-La Roche Inc., Nutley, NJ 07110*

Pseudo-High Affinity Interleukin 2 (IL-2) Receptor Lacks the Third Component That Is Essential for Functional IL-2 Binding and Signaling

By Nobuyoshi Arima,*§ Masanori Kamio,*§ Kazunori Imada,*§
Toshiyuki Hori,§ Toshio Hattori,§ Mitsuru Tsudo,†
Minoru Okuma,* and Takashi Uchiyama§

*From the First Division of Internal Medicine, Faculty of Medicine, Kyoto University, Sakyo, Kyoto 606; the †Department of Internal Medicine, Unitika Central Hospital, Uji, Kyoto 606; and the §Institute for Virus Research, Kyoto University, Sakyo, Kyoto 606, Japan

Summary

Functional studies of the interleukin 2 receptor (IL-2R) of two (ED515-D and Kit225) IL-2-dependent and three (ED515-I, 3T3- $\alpha\beta$ 11, and Hut102) IL-2-independent cell lines were done. All of these cell lines appeared to express high as well as low affinity IL-2R. However, ED515-I and 3T3- $\alpha\beta$ 11, which expressed the IL-2R β chain, did not bind IL-2 at all when IL-2 binding to their IL-2R α chain was blocked with anti-Tac monoclonal antibody, whereas the intermediate affinity binding in ED515-D, Kit225, and Hut102 cells remained. We tentatively called the high affinity IL-2R of the former cells pseudo-high affinity IL-2R. The dissociation constant of pseudo-high affinity IL-2R was higher than that of ordinary high affinity IL-2R. Internalization of cell-bound 125 I-IL-2 into ED515-I and 3T3- $\alpha\beta$ 11 cells was less efficient than that into ED515-D cells. The addition of IL-2 neither promoted cell growth nor upregulated IL-2R α chain expression in ED515-I and 3T3- $\alpha\beta$ 11 cells. Furthermore, tyrosine phosphorylation of the cellular proteins (p120, p98, p96, p54, and p38) was induced or enhanced in response to the addition of IL-2 in ED515-D and Kit225 cells, but not in the cell lines expressing pseudo-high affinity IL-2R. Finally, 125 I-IL-2 crosslinking followed by SDS-PAGE analysis showed an 80-kD band corresponding to p65 + IL-2, in addition to bands corresponding to IL-2R α and β chain + IL-2 in cells bearing ordinary high affinity IL-2R but not in cells with pseudo-high affinity IL-2R. Taken together, we consider that another protein whose molecular mass is approximately 65 kD is functionally important in IL-2 binding and subsequent signal transduction and may be the third component of IL-2R.

IL-2 is a lymphokine produced by T cells that induces proliferation and differentiation of T, B, and NK cells, as well as thymocytes and monocytes. Cells bind IL-2 with three different affinities, designated as low ($K_d = 10^{-8}$ M), intermediate ($K_d = 10^{-9}$ M), and high ($K_d = 10^{-11}$ M) (1-9). Studies using mAbs and affinity crosslinking with radiolabeled IL-2 have identified and characterized the heterodimeric structure of IL-2R (6-15). A low affinity IL-2R consists of an α chain (p55, Tac) (2, 12), and association and dissociation of IL-2 for the low affinity IL-2R are very rapid ($t_{1/2} = 5$ and 6 s, respectively) (16, 17). The IL-2R α chain with a short intracytoplasmic portion does not solely transduce a growth signal (3, 4, 18). In contrast, the IL-2R β chain binds IL-2 with an intermediate affinity when expressed solely and appears to be more important in IL-2 signal transduction. The association and dissociation of IL-2 for the intermediate affinity IL-2R are much slower ($t_{1/2} = 45$ and 290 min, respectively). Because IL-2 binding to the high affinity IL-2R takes

on the characteristics of the low affinity IL-2R for its association and of the intermediate affinity IL-2R for its dissociation ($t_{1/2} = 37$ s and 285 min, respectively), IL-2/high affinity IL-2R complexes are most stable (16, 17). In cell lines that bind IL-2 with only intermediate affinity, doublet bands corresponding to p68 + IL-2 and p75 + IL-2 were detected by crosslinking with radiolabeled IL-2 (19, 20). Hermann et al. (21) and we (22) reported that these two bands differ not only in relative mobility in gel but also in the reactivity with anti-IL-2R β chain Ab, although these two bands had been considered to be due to the degradation of receptor-IL-2 complex or to a different posttranslational modification.

The cDNA cloning of the IL-2R β chain using Mik- β 1 mAb (13) revealed that the IL-2R β chain contains no kinase domains, although it mediates IL-2 internalization and growth signal transduction when expressed on lymphocytes (23). However, transfected fibroblasts expressing the IL-2R β chain did not bind IL-2 (23-25), suggesting that the β chain must

be modified or associated with another chain for IL-2 binding. This possibility is supported by the fact that human NK cells obtained after in vivo IL-2 therapy expressed less IL-2 binding sites than the number of cell surface β chain (26). Saito et al. (27) also showed that the β chain expressed on a nonlymphoid cell line transfected with its cDNA can bind IL-2 when mixed with detergent-solubilized cell membrane of lymphocytes that cannot bind IL-2. A number of investigations have been done in an attempt to identify the third molecule of the IL-2R by chemical crosslinking or immunoprecipitation, and several candidate proteins have been reported (28–33). Although it is presumed to be close to the β chain or to the high affinity IL-2R, the function of the third molecule of IL-2R remains almost unknown.

In the present study, we identified a nontransfected cell line that seemed to lack the third component of IL-2R, and studied the characteristics of its IL-2R to know the role of the third component in IL-2 binding and subsequent signal transduction.

Materials and Methods

Cells and Antibodies. ED515-D is an IL-2-dependent leukemic T cell line established from an adult T cell leukemia patient (34). Kit225 is a human IL-2-dependent cell line derived from a chronic T lymphocytic leukemia patient (35). They were cultured in growth medium containing RPMI 1640, 10% FCS (Gibco Laboratories, Grand Island, NY), 60 mM tobramycin, 2 mM L-glutamine, and 0.5 nM rIL-2 (a gift from Shionogi Research Laboratories, Osaka, Japan), and incubated in IL-2-free medium for 24–48 h before use. ED515-I is a subclone of ED515-D and proliferates independently of IL-2. The 3T3- $\alpha\beta$ 11 cell line was established by transfecting cDNA for both human IL-2R α and β chains into NIH-3T3 cells (24). Hut102, ED515-I, and 3T3- $\alpha\beta$ 11 cells were cultured in growth medium without IL-2. Single cell suspensions of 3T3- $\alpha\beta$ 11 cells were made by treatment with PBS containing 1 mg/ml BSA and 5 mM EDTA, and then cells were resuspended in medium for each examination.

Binding Assay. The binding of 125 I-IL-2 to various types of cell lines at 4°C was measured as described (36). Human rIL-2 (Takeda, Osaka, Japan) was radioiodinated with Enzymobeads (Bio-Rad Laboratories, Richmond, CA) and the specific activity was 35,000 cpm/ng.

Association and Dissociation Kinetics of IL-2 Binding. To study the association kinetics, 4×10^5 cells were incubated with 50 pM of 125 I-IL-2 in 200 μ l of the binding medium containing 10 mg/ml BSA, 1 mg/ml sodium azide, and 25 mM Hepes in RPMI 1640 (pH 7.4) at 4°C. At selected times, cells were centrifuged through a layer of a mixture of 20% olive oil and 80% di-n-butylphthalate, and the radioactivity of the cell pellet was counted by a gamma counter. To study dissociation kinetics, 4×10^5 cells were incubated with 50 pM of 125 I-IL-2 for 60 min at 4°C in 200 μ l of the binding medium. After centrifugation, cells were resuspended in 200 μ l of the binding medium supplemented with 10 nM cold rIL-2. At various times, the cell-associated radioactivity was measured as indicated.

Internalization of 125 I-IL-2. Cells (10^7 cells/ml for ED515-D and ED515-I, 5×10^6 cells/ml for 3T3- $\alpha\beta$ 11) were first incubated at 4°C for 20 min with 200 pM 125 I-IL-2 in RPMI 1640, 25 mM Hepes, pH 7.4, containing 10 mg/ml BSA, and 100 μ M chloroquine (37), then quickly warmed to 37°C in a water bath. At

selected times, 200- μ l aliquots of the cell suspension were centrifuged, and the radioactivity of the supernatant was measured to determine the level of free IL-2. The cell pellets were resuspended in 200 μ l of 0.14 M NaCl, 20 mM glycine-HCl, pH 3.0, containing 1 mg/ml BSA. After 30 s, the cells were centrifuged through a layer of a mixture of 20% olive oil and 80% di-n-butylphthalate, and the radioactivity of the supernatant and the cell pellet was counted to determine the level of internalized or cell surface-bound IL-2 that was resistant or sensitive to the treatment with acidic (pH 3.0) buffer. Nonspecific binding was determined by adding a 1,000-fold excess of unlabeled IL-2.

Western Blotting of Tyrosine-phosphorylated Protein. Tyrosine-phosphorylated proteins were detected by immunoblotting as previously described with minor modifications (38). In brief, aliquots of 10^6 cells per sample were stimulated with rIL-2 (5 nM) and lysed at the indicated times. Whole cell lysates were loaded on to 7.5% SDS-polyacrylamide gels and subjected to electrophoresis. The proteins were transferred to a 0.45- μ m Immobilon-polyvinylidene fluoride membrane (Millipore Corp., Bedford, MA) using a semi-dry transfer apparatus. The PVDF blots were blocked with 5% BSA in blotting buffer (20 mM Tris-HCl buffer, pH 7.4, 150 mM NaCl, 0.05% Tween 20) for 24 h, then incubated with purified rabbit antiphosphotyrosine Ab (Zymed Laboratories, Inc., San Francisco, CA) for 60 min followed by incubation with horseradish peroxidase-conjugated anti-rabbit IgG (Tago, Inc., Burlingame, CA) for 60 min. The membrane was washed in blotting buffer containing 1% BSA, then immunoblots were developed using the ECL System (Amersham Corp., Arlington Heights, IL).

Proliferative Response Assay. After preincubation in IL-2-free medium for 48 h, if necessary, cells were cultured at a density of 10^5 cells/ml in 200 μ l growth medium for 48 h with serially diluted IL-2. Proliferation was measured by the incorporation of 19 kBq of [3 H]TdR (DuPont/NEN Research Products, Boston, MA) per well for the last 4 h of the culture. ED515-I cells in growth medium containing 0.5% FCS were examined in the same manner.

Flow Cytometric Analysis of the Regulation of IL-2R α Chain Expression by IL-2. After preincubation in IL-2-free medium for 24 h, cells were incubated at 3×10^5 cells/ml with or without 1 nM rIL-2 for 18 h at 37°C. Surface-bound IL-2 was removed by washing with acidic buffer for 30 s, and then 10^6 cells were incubated with a saturating quantity of FITC mAbs at 4°C for 30 min. The mean fluorescence intensity (MFI)¹ of the samples was measured using a FACScan[®] (Becton Dickinson Immunocytometry System, Fullerton, CA), and the difference between the MFI of cells stained with anti-Tac mAb and the MFI of cells stained with control mAb was calculated as the quantity of IL-2R α chain expressed on the cells.

Crosslinking Study. Affinity crosslinking was performed as previously described (22).

Results

125 I-IL-2 Binding Studies in the Presence of Anti-Tac mAb 3T3- $\alpha\beta$ 11 cells bear both high and low affinity IL-2R. However, as reported by Tsudo et al. (24), 3T3- $\alpha\beta$ 11 cells do not bind IL-2 in the presence of anti-Tac mAb. We examined 125 I-IL-2 binding to various cell lines in the presence of anti-Tac mAb. ED515-D, Kit225, and Hut102 cells bound IL-2 with an intermediate affinity ($K_d = 1.2$ – 1.6 nM) in the presence of anti-Tac mAb (Table 1). The number of the 125 I-IL-2

¹ Abbreviation used in this paper: MFI, mean fluorescence intensity.

Table 1. 125 I-IL-2 Binding Assay in the Presence or Absence of Anti Tac mAb

Cell line	Types of receptor	Without mAb		With anti-Tac mAb	
		K_d	Sites/cell	K_d	Sites/cell
Kit225	High	pM		pM	
	Intermediate	5	7,000	—*	—
	Low	—	—	1,200	6,000
ED515-D	High	12,000	300,000	—	—
	Intermediate	4	25,000	—	—
	Low	—	—	1,600	27,000
ED515-I	High	13,000	530,000	—	—
	Intermediate	25	16,000	—	—
	Low	—	—	—	—
3T3- $\alpha\beta$ 11	High	10,000	240,000	—	—
	Intermediate	30	15,000	—	—
	Low	—	—	—	—
Hut102	High	9,000	640,000	—	—
	Intermediate	12	4,600	—	—
	Low	—	—	1,200	870
		13,000	260,000	—	—

* —, not detected.

binding sites detectable in the presence of anti-Tac mAb on ED515-D and Kit225 cells was almost equal to that of high affinity IL-2R. Intermediate affinity IL-2 binding in the presence of anti-Tac mAb was also detected on Hut102 cells, although the number of binding sites was unexpectedly smaller. On the other hand, anti-Tac mAb completely abolished IL-2 binding to ED515-I cells. These data indicate that ED515-I and 3T3- $\alpha\beta$ 11 cells appear to have a similar defect in IL-2 binding of the IL-2R β chain. We tentatively called the high affinity IL-2R of these cells (ED515-I and 3T3- $\alpha\beta$ 11) pseudo-high affinity IL-2R to distinguish from the ordinary high affinity IL-2R of ED515-D and Kit 225 cells in this paper.

The Association and Dissociation Kinetics of 125 I-IL-2. The dissociation constant value of the IL-2 binding to the high affinity IL-2R ($K_d = 4$ – 5 pM) of ED515-D and Kit225 cells is apparently smaller than that of the pseudo-high affinity IL-2R ($K_d = 20$ – 30 pM) (Table 1). Since these results were reproducible, we studied the association and dissociation kinetics of IL-2 for these cell lines. When 50 pM 125 I-IL-2 was added to 4×10^5 cells, the amount of radioactivity bound to Kit225 cells was the lowest among the four cell lines, since the number of high affinity IL-2R of Kit225 cells was two- to fourfold smaller than those of other cell lines. The association time courses of the IL-2 binding to the high affinity IL-2R were equal among four cell lines ($t_{1/2}$ association = 1 min) (Fig. 1, A and C). However, IL-2 dissociated rapidly from the pseudo-high affinity IL-2R ($t_{1/2}$ dissociation = 1 min) (Fig. 1 B). Though $\sim 10\%$ of IL-2 dissociated from the high

affinity IL-2R in the first 5 min, the remaining bound IL-2 hardly dissociated during a further 240 min (Fig. 1 D). These data indicate that the higher dissociation constant value of pseudo-high affinity IL-2R is due to the faster dissociation of bound IL-2.

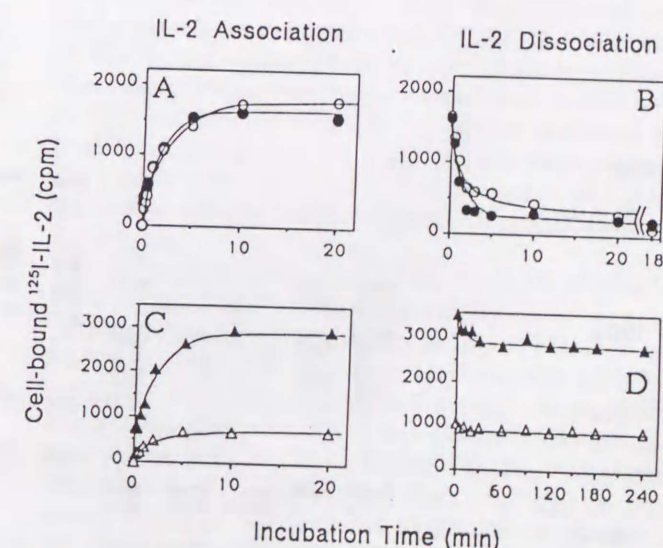


Figure 1. Time courses of association (A and C) and dissociation (B and D) of 125 I-IL-2 in each cell line. Kinetics were studied as described in Materials and Methods on ED515-I cells (O) and 3T3- $\alpha\beta$ 11 cells (●) (A and B), or ED515-D cells (▲) and Kit225 cells (Δ) (C and D).

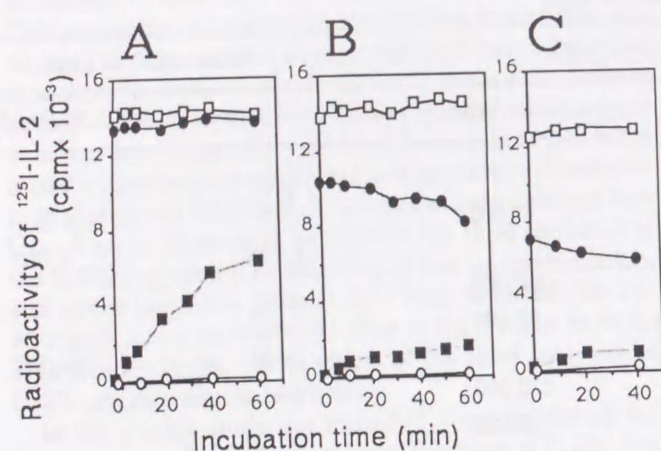


Figure 2. Internalization of ¹²⁵I-IL-2 into ED515-D (A), ED515-I (B), and 3T3-αβ11 cells (C). At each time, the level of radioactivity in pH 3-resistant and internalized ¹²⁵I-IL-2 (■), and pH 3-sensitive and cell surface-bound ¹²⁵I-IL-2 was measured. The total cell-bound ¹²⁵I-IL-2 (●) was measured as the sum of these two fractions. The level of radioactivity in the supernatant was also measured, and the sum of the counts of all three fractions (□) is plotted. After adding excess unlabeled IL-2, pH 3-resistant and internalized ¹²⁵I-IL-2 was measured (○) as nonspecific internalization. The specific internalization of IL-2 is, therefore, the difference between the total and nonspecific internalization.

IL-2 Internalization and IL-2-induced Tyrosine Phosphorylation of the Cellular Proteins. We examined whether cell-bound IL-2 was internalized into the cells after binding to pseudo-high affinity IL-2R. As shown in Fig. 2 A, IL-2 associated with the high affinity IL-2R of ED515-D cells was gradually internalized, and 41% of the total bound IL-2 was internalized at 40 min. Significant internalization of ¹²⁵I-IL-2 was also de-

tected in ED515-I and 3T3-αβ11 cells, but only 14–18% of total bound IL-2 was internalized at 40 min (Fig. 2, B and C). The total cell-bound IL-2 decreased in ED515-I and 3T3-αβ11 cells with increasing incubation time, because the dissociation constant value of pseudo-high affinity IL-2R is higher at 37°C than at 4°C (data not shown).

Next, we performed Western blotting using antiphosphotyrosine Ab to investigate whether IL-2 would increase tyrosine phosphorylation in the cells bearing pseudo-high affinity IL-2R. We consistently observed that IL-2 induced or enhanced tyrosine phosphorylation of proteins expressed as five bands on gels with molecular masses of 120, 98, 96, 54, and 38 kD in ED515-D and Kit225 cells (Fig. 3, lanes 3–8). Similar induction or enhancement of tyrosine phosphorylation of proteins with apparent molecular masses of 98, 96, and 38 kD by IL-2 was detected in Hut102 cells (Fig. 3, lanes 11–13). Tyrosine phosphorylation was detected within 1 min after the addition of IL-2, and the maximal increase was observed 10 min later. IL-2 was reported to induce the tyrosine phosphorylation of the IL-2R β chain (39, 40). The broad and vague band at ~84 kD in ED515-D and Hut102 cells may include the IL-2R β chain. On the other hand, IL-2 induced no increase in tyrosine phosphorylation in ED515-I cells (Fig. 3, lanes 1 and 2). We observed several bands that were constitutively tyrosine phosphorylated in 3T3-αβ11 cells, but IL-2 induced no increase (Fig. 3, lanes 9–10).

The Proliferative Response to IL-2 and the Upregulation of the IL-2R α Chain Expression by IL-2. As shown in Fig. 4 B, ED515-D and Kit225 cells displayed a proliferative response to rIL-2 as measured by [³H]TdR uptake, whereas IL-2 did not enhance [³H]TdR uptake by 3T3-αβ11 and ED515-I cells (Fig. 4 A). Since we supposed that ED515-I cells proliferated at their maximum in medium with 10% FCS and that



Figure 3. Effect of IL-2 on the phosphorylation of proteins at tyrosine residues in ED515-I (lanes 1 and 2), ED515-D (lanes 3–5), Kit225 (lanes 6–8), 3T3-αβ11 (lanes 9 and 10), and Hut102 cells (lanes 11–13) incubated for 0 (lanes 1, 3, 6, 9, and 11), 1 (lanes 4, 7, and 12), or 10 min (lanes 2, 5, 8, 10, and 13) with 2 nM IL-2. The samples were then immunoblotted with Ab to phosphotyrosine as described in Materials and Methods. The arrowheads indicate the position of the 120-, 98-, 96-, 54-, and 38-kD proteins of which tyrosine phosphorylation was increased by IL-2.

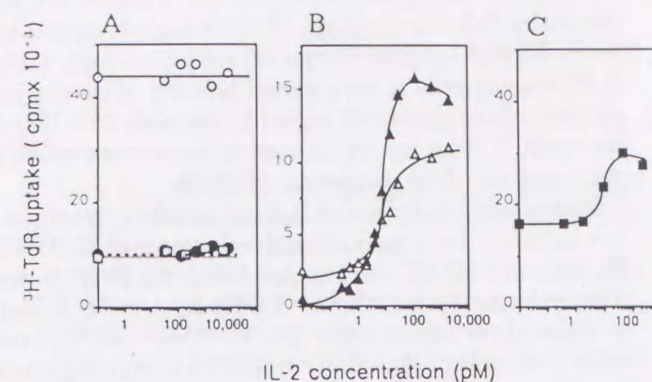


Figure 4. Proliferative response of various cell lines to IL-2. The IL-2 response was monitored by the [³H]TdR uptake in (A) ED515-I cells cultured with 10% FCS (○) or 0.5% FCS (□), and 3T3-αβ11 (●), (B) ED515-D (Δ), Kit225 cells (▲), and (C) Hut102 cells (■).

the addition of IL-2 no longer induced further thymidine incorporation into ED515-I, cell growth-promoting activity of IL-2 in these cells was examined in medium containing a low concentration of FCS (0.5%). However, ED515-I cells also did not respond to IL-2 in this culture condition. In contrast, Hut102 cells, which can be maintained without IL-2, showed IL-2-inducible [³H]TdR uptake in addition to the relatively high basic [³H]TdR uptake (Fig. 4 C).

It was reported that IL-2 upregulates the expression of the IL-2R α chain on T cells (41, 42), and we examined the upregulation of the IL-2R α chain expression by IL-2 stimulation in the cells bearing pseudo-high affinity IL-2R. In ED515-D and Kit225 cells, the addition of IL-2 resulted in a ~1.4–1.6-fold greater expression of the IL-2R α chain as measured by flowcytometric analysis (Table 2). In ED515-I cells, however, the upregulation of IL-2R α chain expression was not found.

¹²⁵I-IL-2 Crosslinking Studies. To explore the molecular properties of the two different types of high affinity IL-2R, a ¹²⁵I-IL-2 crosslinking study with chemical crosslinkers was

Table 2. The Upregulation of the IL-2R α Chain by IL-2

Cell line	MFI*	
	– IL-2	+ IL-2
Kit225	81.8	133.9 (164)†
ED515-D	341.4	486.1 (142)
ED515-I	64.0	60.9 (95)

* Cells were stained with FITC-conjugated anti-Tac mAb or control mAb followed by FACSscan® analysis, and the difference between MFI of cells stained with anti-Tac mAb and that with control mAb was calculated as the quantity of IL-2R α chain expression.

† MFI of each cell line cultured with IL-2 is also shown as the percentage of that cultured without IL-2.

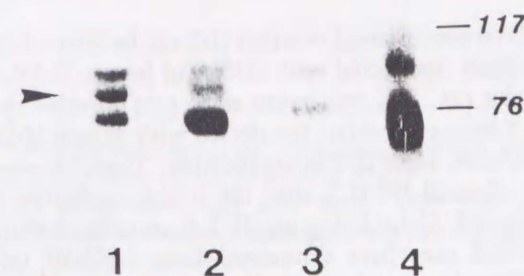


Figure 5. Affinity crosslinking of the IL-2R in various cell lines. Lane 1, ED515-D cells; lane 2, Kit225 cells; lane 3, ED515-I cells; lane 4, 3T3-αβ11 cells. The arrowheads indicate the presence of the middle band seen in ED515-D cells and Kit225 cells.

performed in these cells. Three bands, 72, 80, and 89 kD, were detected in ED515-D and Kit225 cells (Fig. 5, lanes 1 and 2). However, the middle p65 + IL-2 (15-kD) band was not detected in ED515-I and 3T3-αβ11 cells, although both the upper and lower bands corresponding to the IL-2R α and β chains were detected (Fig. 5, lanes 3 and 4).

Discussion

We demonstrated in the present study that ED515-I and 3T3-αβ11 cells have the following characteristics. (a) The IL-2R β chains of the cell lines were not fully functional as intermediate affinity IL-2R, because the anti-Tac mAb completely abolished IL-2 binding to these cell lines. (b) Their high affinity IL-2Rs had higher dissociation constants than those of ED515-D and Kit225. (c) IL-2 bound to them dissociated much faster than that bound to ED515-D and Kit225 cells, although the rate of IL-2 association was similar. (d) IL-2 bound to their high affinity IL-2R was internalized more slowly and to a less extent than in ED515-D and Kit225 cells. (e) IL-2 did not induce or enhance tyrosine phosphorylation of cellular proteins, which was demonstrated in ED515-D, Kit225, and Hut102 cells. (f) IL-2 neither promoted cell growth nor upregulated the IL-2R α chain expression. (g) The protein with a presumed molecular mass of 65 kD and crosslinked with IL-2 was not detected, unlike ED515-D and Kit225 cells.

In this paper we called the high affinity IL-2R of ED515-I and 3T3-αβ11 cells “pseudo-high affinity IL-2R” in comparison with ordinary high affinity IL-2Rs of ED515-D and Kit225 cells. However, we have no evidence that ED515-D and Kit225 cells have exclusively ordinary high affinity IL-2R. The IL-2 dissociation kinetics of high affinity IL-2Rs appeared to be biphasic in ED515-D and Kit225 cells (Fig. 1 D). About 10% of total cell-bound IL-2 that dissociated from the high affinity IL-2Rs of ED515-D and Kit225 cells in the first 5 min may have bound to the pseudo-high affinity IL-2Rs, if any, of these cell lines. Furthermore, Hut102 cells have a smaller number of the intermediate affinity IL-2-binding sites in the presence of anti-Tac mAb than that of high affinity IL-2-binding sites (Table 1). This result may indicate that Hut102 cells bear

a smaller number of the third components necessary for functional intermediate affinity IL-2R than that of the IL-2R β chain.

It has been controversial whether IL-2 can be internalized into fibroblasts transfected with cDNA of human IL-2R α and β chains (24, 25). Minamoto et al. (25) reported that L929 $\alpha\beta$, a mouse fibroblast transfected with human IL-2R α and β chains, lacks IL-2 internalization. They, however, removed unbound ^{125}I -IL-2 after the initial incubation of cells and ligand. Considering our IL-2 dissociation kinetics data, ^{125}I -IL-2 may have dissociated from L929 $\alpha\beta$ cells during cell washing or the incubation steps in their studies. Slower and less internalization of cell-bound IL-2 in ED515-I and 3T3- $\alpha\beta$ 11 cells demonstrated in the present study may suggest the different internalization mechanism or pathway in such cells bearing pseudo-high affinity IL-2R.

The signal transduction pathway of IL-2/IL-2R remains ill defined. The early activation of a tyrosine kinase occurring in response to IL-2 stimulation has been considered to be one of the key events in IL-2 signal transduction (39, 40, 43-45). Although the IL-2R β chain is phosphorylated on tyrosine and serine/threonine residues, the cytoplasmic portion of the IL-2R β chain lacks an obvious kinase domain. Fung et al. (45) reported that some tyrosine kinase physically associated with the IL-2R β chain. It was also reported that several proteins with molecular masses ranging from 38 to 180 kD were phosphorylated on tyrosine upon IL-2 stimulation. We showed that cellular proteins expressed as five bands on gels with molecular masses of 120, 98, 96, 54, and 38 kD were tyrosine phosphorylated upon IL-2 stimulation in ED515-D and Kit225 cells. Since such IL-2-induced tyrosine phosphorylation was undetectable in ED515-I and 3T3- $\alpha\beta$ 11 cells, the pseudo-high affinity IL-2R may be defective in some receptor-associated protein that is indispensable for not only the complete IL-2 binding but also IL-2 signaling. It is noteworthy that Hut102 cells, IL-2 independent in their cell growth and retaining intermediate affinity IL-2 binding in the presence of anti-Tac mAb, showed IL-2-inducible [^3H]TdR uptake and tyrosine phosphorylation in contrast to no response of ED515-I and 3T3- $\alpha\beta$ 11 cells lacking for p65. It is, therefore, unlikely that the loss or absence of p65 is closely associated with the acquisition of IL-2 independency in cell growth and that no response of ED515-I and 3T3- $\alpha\beta$ 11 cells is simply due to the loss of IL-2 dependency.

Hatakeyama et al. (46) reported that an IL-1R β chain mutant with a restricted cytoplasmic serine-rich region (amino acids 267-322) bound IL-2 with coexpression of the IL-2R

α chain, followed by internalization, but its growth was not affected by IL-2. The similarity of the biological response between this IL-2R β chain mutant cell and pseudo-high affinity IL-2R-bearing cells as we reported here may show the importance of the serine-rich region (amino acids 267-322) of the IL-2R β chain for the interaction between the IL-2R β chain and the third component of IL-2R.

Studies using radiolabeled IL-2 and crosslinking reagents have indicated that a series of additional proteins of 22, 35-40, 90, 100, and 115 kD are associated with the IL-2R α and β chains in the murine cells (28-30). However, it was difficult to detect them reproducibly. The 80-90-kD non-Tac band easily detectable in the affinity crosslinking was not a single band, but a doublet (19, 20). As we recently reported, the lower band of the doublet appears not to be the IL-2R β chain, because polyclonal anti-IL-2R β chain Ab cannot react with it (22). By using limited proteolysis, Hermann and Diamantstein also reported that this lower doublet band differs from the upper one, although they both displayed a high homology. As shown in Fig. 5, the lower band of the doublet was not detected in either ED515-I or 3T3- $\alpha\beta$ 11 cells, suggesting that the key molecule for functional IL-2R is the 65-kD molecule (p65). This is supported by the recent report by Takeshita et al. (33). They showed that the amount of p64 coprecipitated with the IL-2R β chain in the presence of IL-2 was proportional to the number of the IL-2 binding sites, but not to that of the IL-2R β chain. Our observation that p65 could be crosslinked with IL-2 in cells bearing high affinity IL-2R but not in cells bearing pseudo-high affinity IL-2R leads to two possible explanations with respect to the role of p65 in IL-2 binding. One is that p65 directly binds IL-2 as the supporter of the IL-2R β chain, and the other is that p65, located close to the IL-2R β chain, exerts a steric effect on the β chain without directly binding IL-2. The latter seems likely because the MT- β 7 cell, which is MT-1 cell transfected with the human IL-2R β chain (47), bound IL-2 with an intermediate affinity when examined in the presence of anti-Tac mAb (data not shown), suggesting that MT- β 7 cells have the third component of IL-2R. Parental MT-1 cells, however, bind IL-2 with only low affinity and IL-2 cannot be cross-linked with p65 in MT-1 cells (22). These data suggest that the third component cannot bind IL-2 without the help of the IL-2R β chain.

Finally, it would be of interest to determine whether there exist such cells having pseudo-high affinity IL-2R in vivo and which biological responses they, if present, are involved in.

We thank Dr. Maeda (Kyoto University, Kyoto, Japan) for ED515-D and ED515-I. We also thank Drs. Karasuyama and Kubo (Tokyo University, Tokyo, Japan) for the BCMGNeo vector.

This work was supported by Special Coordination Funds of the Science and Technology Agency of the Japanese Government and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

Address correspondence to Takashi Uchiyama, Institute for Virus Research, Kyoto University, 53 Shogoin-Kawaramachi, Sakyo, Kyoto 606, Japan.

Received for publication 26 May 1992.

References

1. Robb, R.J., A. Munck, and K.A. Smith. 1981. T cell growth factor receptors: quantitation, specificity, and biological relevance. *J. Exp. Med.* 154:1455.
2. Leonard, W.J., J.M. Depper, T. Uchiyama, K.A. Smith, T.A. Waldmann, and W.C. Greene. 1982. A monoclonal antibody that appears to recognize the receptor for human T cell growth factor; partial characterization of the receptor. *Nature (Lond.)* 300:267.
3. Nikaido, T., A. Shimizu, N. Ishida, H. Sabe, K. Teshigawara, M. Maeda, T. Uchiyama, J. Yodoi, and T. Honjo. 1984. Molecular cloning of cDNA encoding human interleukin-2 receptor. *Nature (Lond.)* 311:631.
4. Leonard, W.J., J.M. Depper, G.R. Crabtree, S. Rudikoff, J. Pumphrey, R.J. Robb, M. Kronke, P.B. Svetlik, N.J. Peffer, T.A. Waldmann, and W.C. Greene. 1984. Molecular cloning and expression of cDNAs for the human interleukin-2 receptor. *Nature (Lond.)* 311:626.
5. Robb, R.J., W.C. Greene, and C.M. Rusk. 1984. Low and high affinity cellular receptors for interleukin 2: implications for the level of Tac antigen. *J. Exp. Med.* 160:1126.
6. Sharon, M., R.D. Klausner, B.R. Cullen, R. Chizzonite, and W.J. Leonard. 1986. Novel interleukin-2 receptor subunit detected by cross-linking under high affinity conditions. *Science (Wash. DC)* 234:859.
7. Tsudo, M., R.W. Kozak, C.K. Goldman, and T.A. Waldmann. 1986. Demonstration of a non-Tac peptide that binds interleukin 2: a potential participant in a multichain interleukin 2 receptor complex. *Proc. Natl. Acad. Sci. USA* 83:9694.
8. Teshigawara, K., H. Wang, K. Kato, and K.A. Smith. 1987. Interleukin 2 high-affinity receptor expression requires two distinct binding proteins. *J. Exp. Med.* 165:223.
9. Robb, R.J., C.M. Rusk, J. Yodoi, and W.C. Greene. 1987. Interleukin 2 binding molecule distinct from Tac protein: analysis of its role in formation of high-affinity receptors. *Proc. Natl. Acad. Sci. USA* 84:2002.
10. Tsudo, M., R.W. Kozak, C.K. Goldman, and T.A. Waldmann. 1987. Contribution of a p75 interleukin 2 binding peptide to a high-affinity interleukin 2 receptor complex. *Proc. Natl. Acad. Sci. USA* 84:4215.
11. Lowenthal, J.W., and W.C. Greene. 1987. Contrasting interleukin 2 binding properties of the α (p55) and β (p70) protein subunits of the human high-affinity interleukin 2 receptor. *J. Exp. Med.* 166:1156.
12. Uchiyama, T., S. Broder, and T.A. Waldmann. 1981. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. *J. Immunol.* 126:1393.
13. Tsudo, M., F. Kitamura, and M. Miyasaka. 1989. Characterization of the interleukin 2 receptor β chain using three distinct monoclonal antibodies. *Proc. Natl. Acad. Sci. USA* 86:1982.
14. Takeshita, T., Y. Goto, K. Tada, K. Nagata, H. Asao, and K. Sugamura. 1989. Monoclonal antibody defining a molecule possibly identical to the p75 subunit of interleukin 2 receptor. *J. Exp. Med.* 169:1323.
15. Kamio, M., T. Uchiyama, N. Arima, K. Itoh, T. Ishikawa, T. Hori, and H. Uchino. 1990. Role of α chain-IL-2 complex in the formation of the ternary complex of IL-2 and high-affinity IL-2 receptor. *Int. Immunol.* 2:521.
16. Wang, H.M., and K.A. Smith. 1987. The interleukin 2 receptor: functional consequences of its bimolecular structure. *J. Exp. Med.* 166:1055.
17. Lowenthal, J.W., and W.C. Greene. 1987. Contrasting interleukin 2 binding properties of the α (p55) and β (p70) protein subunits of the human high-affinity interleukin 2 receptor. *J. Exp. Med.* 166:1156.
18. Greene, W.C., R.J. Robb, P.B. Svetlik, C.M. Rusk, J.M. Depper, and W.J. Leonard. 1985. Stable expression of cDNA encoding the human interleukin 2 receptor in eukaryotic cells. *J. Exp. Med.* 162:363.
19. Robb, R.J., C.M. Rusk, J. Yodoi, and W.C. Greene. 1987. Interleukin 2 binding molecule distinct from the Tac protein: Analysis of its role in formation of high-affinity receptors. *Proc. Natl. Acad. Sci. USA* 84:2002.
20. Sharon, M., J.P. Siegel, G. Tosato, J. Yodoi, T.L. Gerrard, and W.J. Leonard. 1988. The human interleukin 2 receptor β chain (p70): direct identification, partial purification, and patterns of expression on peripheral blood mononuclear cells. *J. Exp. Med.* 167:1265.
21. Herrmann, T., and T. Diamantstein. 1988. The human intermediate-affinity interleukin 2 receptor consists of two distinct, partially homologous glycoproteins. *Eur. J. Immunol.* 18:1051.
22. Kamio, M., N. Arima, M. Tsudo, K. Imada, M. Okuma, and T. Uchiyama. 1992. The third molecule associated with interleukin 2 receptor α and β chain. *Biochem. Biophys. Res. Commun.* 184:1288.
23. Hatakeyama, M., M. Tsudo, S. Minamoto, T. Kono, T. Doi, T. Miyata, M. Miyasaka, and T. Taniguchi. 1989. Interleukin-2 receptor β chain gene: generation of three receptor forms by cloned human α and β chain cDNAs. *Science (Wash. DC)* 244:551.
24. Tsudo, M., H. Karasuyama, F. Kitamura, T. Tanaka, S. Kubo, Y. Yamamura, T. Tamatani, M. Hatakeyama, T. Taniguchi, and M. Miyasaka. 1990. The IL-2 receptor β -chain (p70): ligand binding ability of the c-DNA-encoding membrane and secreted forms. *J. Immunol.* 145:599.
25. Minamoto, S., H. Mori, M. Hatakeyama, T. Kono, T. Doi, T. Ide, T. Uede, and T. Taniguchi. 1990. Characterization of the heterodimeric complex of human IL-2 receptor α - β chains reconstituted in a mouse fibroblast cell line, L929. *J. Immunol.* 145:2177.
26. Voss, S.D., R.J. Robb, G. Weil-Hillman, J.A. Hank, K. Sugamura, M. Tsudo, and P.M. Sondel. 1990. Increased expression of the interleukin 2 (IL-2) receptor β chain (p70) on CD56 $^{+}$ natural killer cells after in vivo IL-2 therapy: p70 expression does not alone predict the level of intermediate affinity IL-2 binding. *J. Exp. Med.* 172:1101.
27. Saito, Y., H. Tada, H. Sabe, and T. Honjo. 1991. Biochemical evidence for a third chain of the interleukin-2 receptor. *J. Biol. Chem.* 266:22186.
28. Hermann, T., and T. Diamantstein. 1988. The high affinity interleukin 2 receptor: evidence for three distinct polypeptide

- chains comprising the high affinity interleukin 2 receptor. *Mol. Immunol.* 25:1201.
29. Saragovi, H., and T.R. Malek. 1990. Evidence for additional subunits associated to the mouse interleukin 2 receptor p55/p75 complex. *Proc. Natl. Acad. Sci. USA.* 87:11.
 30. Sharon, M., J.R. Gnarr, and W.J. Leonard. 1990. A 100-kilodalton protein is associated with the murine interleukin 2 receptor: biochemical evidence that p100 is distinct from the α and β chains. *Proc. Natl. Acad. Sci. USA.* 87:4869.
 31. Colamonici, O.R., L.M. Neckers, and A. Rosolen. 1990. Putative γ -subunit of the IL-2 receptor is detected in low, intermediate, and high affinity IL-2 receptor-bearing cells. *J. Immunol.* 145:155.
 32. Takeshita, T., H. Asao, J. Suzuki, and K. Sugamura. 1990. An associated molecule, p64, with high-affinity interleukin 2 receptor. *Int. Immunol.* 2:477.
 33. Takeshita, T., K. Ohtani, H. Asao, S. Kumaki, M. Nakamura, and K. Sugamura. 1982. An associated molecule, p64, with IL-2 receptor β chain: its possible involvement in the formation of the functional intermediate-affinity IL-2 receptor complex. *J. Immunol.* 148:2154.
 34. Maeda, M., A. Shimizu, K. Ikuta, H. Okamoto, M. Kashihara, T. Uchiyama, T. Honjo, and J. Yodoi. 1985. Origin of human T-lymphotropic virus I-positive T cell lines in adult T cell leukemia: Analysis of T cell receptor gene rearrangement. *J. Exp. Med.* 162:2169.
 35. Hori, T., T. Uchiyama, M. Tsudo, H. Umadome, H. Ohno, S. Fukuhara, K. Kita, and H. Uchino. 1987. Establishment of an interleukin 2-dependent human T cell line from a patient with T cell chronic lymphocytic leukemia who is not infected with human T cell leukemia/lymphoma virus. *Blood.* 70:1069.
 36. Arima, N., M. Kamio, M. Okuma, G. Ju, and T. Uchiyama. 1991. The IL-2 receptor α -chain alters the binding of IL-2 to the β -chain. *J. Immunol.* 147:3396.
 37. Robb, R.J., and W.C. Greene. 1987. Internalization of interleukin 2 is mediated by the β chain of the high-affinity interleukin 2 receptor. *J. Exp. Med.* 165:1201.
 38. Dibirdik, I., M.C. Langlie, J.A. Ledbetter, L. Tuel-Ahlgren, V. Obuz, K.G. Waddick, K. Gajl-Peczalska, G.L. Scieven, and F.M. Uckun. 1991. Engagement of interleukin-7 receptor stimulates tyrosine phosphorylation, phosphoinositide turnover, and clonal proliferation of human T-lineage acute lymphoblastic leukemia cells. *Blood.* 78:564.
 39. Asao, H., T. Takeshita, M. Nakamura, K. Nagata, and K. Sugamura. 1990. Interleukin-2 (IL-2)-induced tyrosine phosphorylation of IL-2 receptor p75. *J. Exp. Med.* 171:637.
 40. Mills, G.B., C. May, M. McGill, M. Fung, M. Baker, R. Sutherland, and W.C. Greene. 1990. Interleukin 2-induced tyrosine phosphorylation: interleukin 2 receptor β is tyrosine phosphorylated. *J. Biol. Chem.* 265:3561.
 41. Reem, G.H., and N.H. Yeh. 1984. Interleukin 2 regulates expression of its receptor and synthesis of gamma interferon by human T lymphocytes. *Science (Wash. DC).* 225:429.
 42. Smith, K.A., and D.A. Cantrell. 1985. Interleukin 2 regulates its own receptors. *Proc. Natl. Acad. Sci. USA.* 82:864.
 43. Saltzmann, E.M., R.R. Thom, and J.E. Casnellie. 1988. Activation of a tyrosine protein kinase is an early event in the stimulation of T lymphocytes by interleukin-2. *J. Biol. Chem.* 263:6956.
 44. Farrar, W.L., and D.K. Ferris. 1989. Two-dimensional analysis of interleukin 2-regulated tyrosine kinase activation mediated by the p70-75 β subunit of the interleukin 2 receptor. *J. Biol. Chem.* 264:12562.
 45. Fung, M.R., R.M. Searce, J.A. Hoffman, N.J. Peffer, S.R. Hammes, J.B. Hosking, R. Schmandt, W.A. Kuziel, B.F. Haynes, G.B. Mills, and W.C. Greene. 1991. A tyrosine kinase physically associates with the β -subunit of the human IL-2 receptor. *J. Immunol.* 147:1253.
 46. Hatakeyama, M., H. Mori, T. Doi, and T. Taniguchi. 1989. A restricted cytoplasmic region of IL-2 receptor β chain is essential for growth signal transduction but not for ligand binding and internalization. *Cell.* 59:837.
 47. Tsudo, M., H. Karasuyama, F. Kitamura, Y. Nagasaka, T. Tanaka, and M. Miyasaki. 1989. Reconstitution of a functional IL-2 receptor by the β chain cDNA: a newly acquired receptor transduces negative signal. *J. Immunol.* 143:4039.